

WHAT IS CLAIMED:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a cynomolgus monkey Dickkopf-4 (cDkk-4) protein which has an amino acid sequence as set forth in SEQ ID NO:2.
2. The isolated nucleic acid of Claim 1 wherein the nucleic acid is a DNA.
- 10 3. The isolated nucleic acid of Claim 1 wherein the nucleic acid is an RNA.
4. The isolated nucleic acid of Claim 1 wherein the nucleic acid is a cDNA.
- 15 5. The isolated nucleic acid of Claim 1 wherein the nucleic acid has a nucleotide sequence as set forth in SEQ ID NO:1.
6. An isolated protein comprising an amino acid sequence as set forth 20 in SEQ ID NO:2.
7. An antibody which binds a protein comprising an amino acid sequence as set forth in SEQ ID NO:2.
- 25 8. A vector comprising a nucleic acid encoding a cynomolgus monkey Dickkopf-4 (cDkk-4) protein which has an amino acid sequence as set forth in SEQ ID NO:2.
9. A cell comprising a nucleic acid encoding a cynomolgus monkey Dickkopf-4 (cDkk-4) protein which has an amino acid sequence as set forth in SEQ ID NO:2 30 wherein the nucleic acid is operably linked to a heterologous promoter.
10. A method for producing a cynomolgus monkey Dickkopf-4 (cDkk-4) protein which binds a low-density lipoprotein receptor protein 5 (LRP5) comprising:
 - (a) providing a nucleic acid encoding the cDkk-4 protein 35 operably linked to a heterologous promoter;

(b) introducing the nucleic acid into a cell to produce a recombinant cell; and

(c) culturing the recombinant cell under conditions which allows expression of the cDkk-4 protein to produce the cDkk-4.

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11. A method for determining whether an analyte is an antagonist of Dickkopf 4 (Dkk-4) comprising:

(a) providing a polypeptide comprising the extracellular domain of a Dkk-4 receptor;

10 (b) contacting the polypeptide with a cynomolgus monkey Dkk-4 (cDkk-4) and the analyte; and

(c) determining whether binding of the cDkk-4 to the polypeptide is decreased in the presence of the analyte, wherein a decrease in the binding indicates that the analyte is an cDkk-4 antagonist.

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12. The method of claim 11, wherein the Dkk-4 receptor is low-density lipoprotein receptor related protein 5 (LRP5) or low density lipoprotein receptor related protein 6 (LRP6).

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13. The method of claim 11, wherein the Dkk-4 receptor is kremen1 or kremen2.

14. The method of Claim 11 wherein the cDkk-4 is labeled.

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15. The method of Claim 11 wherein the cDkk-4 is a fusion protein.

16. A method for determining whether an analyte is an antagonist of Dickkopf-4 (Dkk-4) protein, which comprises:

30 (a) providing a recombinant cell which produces one or more Dkk-4 receptors;

(b) introducing a reporter expression vector into the recombinant cell which comprises a reporter gene operably linked to a promoter responsive to Wnt-mediated signal transduction to provide a second recombinant cell;

35 (c) exposing the second recombinant cell to the analyte and to a cynomolgus monkey Dkk-4 (cDkk-4); and

(d) measuring expression of the reporter gene, wherein an increase in expression of the reporter gene in the presence of the analyte relative to expression of the reporter gene in the absence of the analyte indicates that the analyte is a Dkk-4 antagonist.

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17. The method of Claim 16 wherein the one or more Dkk-4 receptors are selected from the group consisting of low-density lipoprotein receptor protein 5 (LRP5), low-density lipoprotein receptor protein 6 (LRP6), kremen1, kremen2, and combinations thereof.

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18. The method of Claim 16 wherein the promoter comprises one or more lymphoid enhancer factor/T cell factor (TCF/LEF) binding sites.

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19. The method of Claim 16 wherein the cDkk-4 is provided exogenously as an isolated cDkk-4 protein or as a component of a medium obtained from a culture comprising a second recombinant cell which expresses the cDkk-4.

20. The method of Claim 16 wherein the cDkk-4 is provided by cotransfected the second recombinant cell with an expression vector encoding the cDkk-4.

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21. The method of Claim 16 wherein a Wnt ligand is provided exogenously to the second recombinant cell in step (c) as an isolated Wnt ligand or as a component of a medium obtained from a culture comprising a second recombinant cell which expresses the Wnt ligand.

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22. The method of Claim 16 wherein a Wnt ligand is provided to the second recombinant cell by cotransfected the second recombinant cell with an expression vector encoding the Wnt ligand.

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23. A method for determining whether an analyte interferes with binding of Dickkopf-4 (Dkk-4) protein to a Dkk-4 receptor, which comprises:

(a) providing a recombinant cell which expresses the Dkk-4 receptor;

35 (b) culturing the recombinant cell in a culture medium which contains a cynomolgus monkey Dkk-4 (cDkk-4) protein and the analyte; and

(c) measuring the cDkk-4 bound to the cDkk-4 receptor, wherein a decrease in the cDkk-4 protein bound to the Dkk-4 receptor in the presence of the analyte relative to cDkk-4 protein bound in the absence of the analyte indicates that the analyte interferes with the binding of the Dkk-4 protein to the Dkk-4 receptor.

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24. The method of Claim 23 wherein the Dkk-4 receptor is selected from the group consisting of low-density lipoprotein receptor protein 5 (LRP5), low-density lipoprotein receptor protein 6 (LRP6), kremen1, kremen2, and combinations thereof.

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25. The method of Claim 23 wherein the cDkk-4 is labeled.

26. The method of Claim 23 wherein the cDkk-4 is a fusion protein.

15 27. A method of identifying an analyte that induces Wnt signaling comprising:

(a) transfecting a recombinant cell expressing one or more Dkk-4 receptors with a reporter gene operably linked to a promoter responsive to Wnt-mediated signal transduction;

20 (b) exposing the cells to an analyte, cynomolgus monkey Dkk-4 (cDkk-4), and a Wnt ligand;

(c) measuring expression of the reporter gene, wherein an increase in expression of the reporter gene in the presence of the analyte relative to expression of the reporter gene in the absence of the analyte indicates that the analyte induces the Wnt signaling.

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28. The method of Claim 27 wherein the one or more Dkk-4 receptors are selected from the group consisting of low-density lipoprotein receptor protein 5 (LRP5), low-density lipoprotein receptor protein 6 (LRP6), kremen1, kremen2, and combinations thereof.

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29. The method of Claim 27 wherein the promoter comprises one or more lymphoid enhancer factor/T cell factor (TCF/LEF) binding sites.

30. The method of Claim 27 wherein the cDkk-4 is provided exogenously as an isolated cDkk-4 protein or as a component of a medium obtained from a culture comprising a second recombinant cell which expresses the cDkk-4.

5 31. The method of Claim 27 wherein the cDkk-4 is provided by cotransfected the recombinant cell with an expression vector encoding the cDkk-4.

10 32. The method of Claim 27 wherein the Wnt ligand is provided exogenously as an isolated Wnt ligand or as a component of a medium obtained from a culture comprising a second recombinant cell which expresses the Wnt ligand.

33. The method of Claim 27 wherein the Wnt ligand is provided by cotransfected the recombinant cell with an expression vector encoding the Wnt ligand.

15 34. A method for determining whether a compound inhibits Dickkopf 4 (Dkk-4) protein suppression of osteoblast differentiation comprising:

(a) providing pluripotent cells which can be induced to differentiate along an osteoblast lineage;
20 (b) transfecting the cells with a first expression vector which expresses a cynomolgus monkey Dkk-4 (cDkk-4) protein, a second expression vector, which expresses low-density lipoprotein receptor protein (LRP), and a third expression vector which expresses Wnt protein;

(c) incubating the cells in a medium containing the analyte for a time sufficient for expression of the cDkk-4 protein, LRP, and Wnt protein; and

25 (d) measuring expression of one or more osteoblastic markers wherein expression of the one or more markers indicates that the analyte inhibits cDkk-4 suppression of osteoblast differentiation.

35. The method of Claim 34 wherein the pluripotent cells are pluripotent marrow stromal cells or pluripotent mesenchymal cells.

36. The method of Claim 34 wherein the pluripotent cells are selected from the group consisting of ST2 cells and C3H10T1/2 cells.

37. The method of Claim 34 wherein the one or more osteoblastic markers are selected from the group consisting of alkaline phosphatase, Bglap, and Runx2.